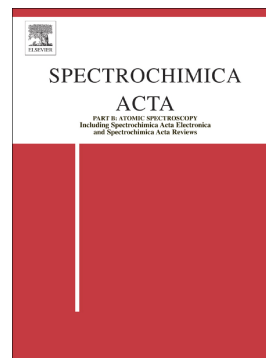


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Selenium analysis by an integrated microwave digestion-needle trap device with hydride sorption on carbon nanotubes and electrothermal atomic absorption spectrometry determination

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**SELENIUM ANALYSIS BY AN INTEGRATED MICROWAVE
DIGESTION-NEEDLE TRAP DEVICE WITH HYDRIDE SORPTION
ON CARBON NANOTUBES AND ELECTROTHERMAL ATOMIC
ABSORPTION SPECTROMETRY DETERMINATION.**

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Abstract

An integrated microwave assisted digestion (MW-AD) – needle trap device (NTD) for selenium determination in grape pomace samples is presented. The NTD was filled with oxidized multiwall carbon nanotubes (oxMWCNTS) where Se hydrides were preconcentrated. Determination was carried out by flow injection-electrothermal atomic absorption spectrometry (FI-ETAAS). The variables affecting the system were established by a multivariate design (Plackett Burman), indicating that the following variables significantly affect the system: sample amount, HNO₃ digestion solution concentration, NaBH₄ volume and elution volume. A Box-Behnken design was implemented to determine the optimized values of these variables. The system improved Se atomization in the graphite furnace, since only trapped hydrides reached the graphite furnace, and the pyrolysis stage was eliminated according to the aqueous matrix of the eluate. Under optimized conditions the system reached a limit of quantification of 0.11 µg kg⁻¹, a detection limit of 0.032 µg kg⁻¹, a relative standard deviation of 4% and a preconcentration factor (PF) of 100, reaching a throughput sample of 5 samples per hour. Sample analysis show Se concentrations between 0.34 ± 0.03 µg kg⁻¹ to 0.48 ± 0.03 µg kg⁻¹ in grape pomace. This system provides minimal reagents and sample consumption, eliminates discontinuous stages between samples processing reaching a simpler and faster Se analysis.

Keywords: Selenium; Needle Trap Device; Carbon nanotubes; Hydride trapping; Electrothermal Atomic Absorption Spectrometry.

1. Introduction.

Selenium is an essential element of important metabolic pathways, including defense through the immune system, thyroid hormone metabolism, reproductive development and antioxidant defense [1]. In the environment, selenium is present in both soil and water, and can be mobilized through plants or aquatic organisms thus entering the food chain [2]. Vine production offers a byproduct called pomace that includes skins of grape and seeds [3]. An adequate use of agroindustrial residues, such as pomace, could have an impact on agricultural productivity and soil fertility through composting [4]. Therefore, the environmental impact of this practice should be assessed by determining trace elements in pomace with essential and toxic characteristics, like selenium [5].

In order to monitor selenium in pomace samples it is necessary to develop analytical methods with appropriate detection levels. Sample preparation plays a decisive role throughout the analytical procedure, especially for analysis of solid environmental samples, characterized by complex matrices and the presence of trace and ultratraces of analytes [6, 7]. The microwave assisted wet digestion (MW-AD) presents great advantages over classical methods, allowing a reduction of the extraction time, less use of reagents and the possibility of on-line coupling to other analytical stages [8-10]. Preconcentration strategies for volatile species generated during hydride generation (HG) processes have been described, resulting in gas-phase trapping techniques [11-13]. HG by sodium tetrahydroborate shows advantages like analyte separation from the matrix, eliminating interferences and increasing sensitivity [14]. Not all species of metals can react with NaBH_4 and form hydrides, for total Se determination, only Se (IV) forms the volatile compound, H_2Se . Because of this, the transformation of the different selenium compounds to their Se

(IV) species is unavoidable. It is possible to reduce Se (VI) to Se (IV) by the use of hydrochloric acid 6 M, or 1 or 2 M HCl with heating [15].

Trace analysis where a pretreatment of the sample is required, like MW-AD and HG, can be integrated to preconcentration procedures by a novel approach: needle trap devices (NTD) [16]. The axis of the NTD consists of a sorbent material, packed inside a stainless steel needle. In this methodology the sample passes over the sorbent, where the analytes are trapped and then subjected to thermal or solvent desorption [17]. Its main benefits are that the extraction phase is much larger and has an exhaustive extraction nature, allowing the complete extraction of the analytes present in the sample. It also presents greater robustness and ease of handling during sampling and desorption. Since elution can be achieved with volumes in the microliters order, NTD can be coupled on line to sensible techniques like electrothermal atomic absorption spectrometry (ETAAS) [16, 18-20].

Recently, Bagheri *et al.* developed a methodology where they used for first time a sorbent based on CNTs in a NTD for microextraction of polycyclic aromatic hydrocarbons from aquatic media [21]. The use of NTCs as NTD sorbents has generated great interest in the scientific community, which is related to their unique properties, such as chemical stability, durability and corrosion resistance [22]. In addition, these materials have a high surface area and can be easy to process. It should be noted that the preferential use of MWCNTs as sorbents is evident, which may be due to the fact that SWCNTs present more resistance to acid treatments [23]. Thus, the acid oxidation conditions required for functionalization are less aggressive for the MWCNTs [24]. Furthermore, MWCNTs have

superior physical properties such as increased strength and thermal and electrical conductivity.

In the present work a method was developed to analyze selenium in grape pomace samples by integrating MW-AD, HG and NTD in a FI system. Selenium hydrides were micro-preconcentrated on oxidized MWCNTs (oxMWCNTs) with ETAAS determination. The study of the different variables affecting the system was performed by a multivariate experimental design. After optimization of these variables, the analytical results as well as the validation studies on grape pomace samples were successfully evaluated.

2. Experimental

2.1. Standard and reagents.

All reagents used were of analytical grade, and no presence of Se was detected in the working range. The MWCNTs were obtained from Sigma-Aldrich (St. Louis, USA). A standard stock solution (100.0 mg L^{-1}) of Se was prepared by dissolving an appropriate amount of Na_2SeO_3 (Sigma, St. Louis, USA) in 5% HNO_3 . The diluted working solutions were prepared daily by serial dilutions of the stock solution. A solution of 1% (w/v) sodium tetrahydroborate (Aldrich Chemical Co., St. Louis, USA, 98%) was prepared in 0.5% (w/v) sodium hydroxide solution and filtered through filter paper Whatman No. 42 to remove undissolved solids. This solution was prepared daily. H_2SO_4 , HNO_3 , H_2O_2 , HCl used for the functionalization of MWCNTs as well as the digestion and treatment of samples were from Merck, Darmsdat, Germany.

2.2. Apparatus/ Instrumentation.

Determination of Se concentration was carried out on a Shimadzu Model AA-7000 atomic absorption spectrometer (Tokyo, Japan) equipped with a background correction system employing a continuum source, a GFA-EX7 electrothermal atomizer, and an ASC-7000 auto sampler. L'vov graphite tubes (Shimadzu, Tokyo, Japan) were used in all experiments. A selenium hollow-cathode lamp (Hamamatsu, Photonics K.K., Japan) was employed as radiation source at 196.0 nm with a slit of 1.0 nm. The ETAAS instrumental and operating conditions are listed in Table 1. The graphite tubes were pre-treated three times with the iridium modifier as follows: 50 μ L of 1000 g L⁻¹ Ir stock solution were injected into the graphite tube and heated according to the temperature program given in Table S1 (Supplementary material) [25]. The temperature of pyrolysis was optimized from 0°C to 700 °C.

A domestic microwave oven Samsung model G245C (Seoul, South Korea) of 2450 MHz frequency and operating at a maximum exit power of 1500 W was employed for MW-AD. For validation, a digestion was carried out in a laboratory microwave digester (Milestone, Sorisole, Italy). Digestion was performed according to the manufacturer indications: 0.5 g of pomace samples were weighed and placed in individual microwave reactors. The aliquots were treated with 7 mL concentrated HNO₃ and 1 mL H₂O₂. Reactors were placed in the digester at a ramp temperature of 10 min up to 200 °C and hold for 10 more minutes. The employed microwave power was up to 1000 W.

Gilson Minipuls 3 peristaltic pumps (Villiers, Le-Bell, France) and Tygon-type pump tubes (Ismatec, Cole-Parmer Instrument Company, Niles, IL, USA) were

employed to propel sample, eluent and reagents. Headspace vials (20 mL) and accessories were obtained from Perkin Elmer (Thornhill, Canada). These vials were used for sample digestion, as well as for HG. All unions were sealed to avoid gas losses.

The Design Expert® 7.0 software package (Stat-Ease Inc., Minneapolis, USA) was used for experimental designs, data analysis and response surfaces.

2.3. MWCNTs oxidation.

To achieve CNTs functionalization, 0.1 g of MWCNT were weighed and placed in a 500 mL balloon. Then, 200 mL of oxidizing mixture H_2SO_4 : HNO_3 (3: 1) were added and covered with a detachment tube. After this, the balloon was placed in an ultrasonic bath and was sonicated for 1 hour. Then the mixture was placed in a thermostated bath (55 °C) for 1 hour. Then 200 mL of Milli-Q water were added and the mixture was allowed to cool at room temperature. By centrifugation at 4000 rpm, the acid was removed and washed with pure water, until neutral pH is reached. Finally, oxidized MWCNTs were dried at 70 °C for 12 hours and weighed. OxMWCNTs were chosen for Se hydride retention according to previous works were these show a higher hydride retention efficiency [16, 17].

2.4. NTD preparation.

The NTD was built using a stainless steel bevel needle with a length of 40 mm and an internal diameter of 0.5 mm. To avoid sorbent losses, the needle was pre-

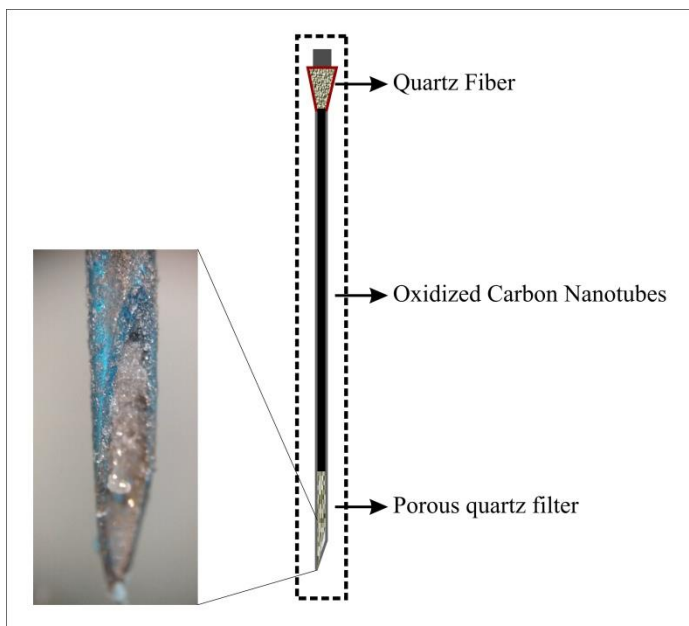


Figure 1: Schematic of the packed NTD.

conditioned by placing glass wool in the needle bevel, applying heat to partially fuse the glass wool to generate a porous stopper. The needle was filled by preparing an aqueous suspension with 4 mg of oxMWCNT, which was introduced into the syringe using the FI system. Thus, the porous stopper retained CNTs, allowing the

packaging. Finally glass wool was placed in the upper side of the needle (Figure 1).

2.5. Samples.

Grape pomace samples were obtained from wine industries of Valley of Tulum, San Juan, Argentina. They were dried at room temperature, pulverized and homogenized. Samples were stored in sterilized PVC containers until sample treatment.

2.6. Procedure.

For determination of selenium ultratraces in grape pomace samples, a closed system is proposed as shown in Figure 2 (MW Digestion Stage/HG/NTD).

In a first stage (DIGESTION), 100 mg of solid sample were weighed and placed in a glass headspace vial and sealed with a Teflon[®] septum. After this, the vial was

introduced into the microwave oven. A security valve consisting in a capillary tube was inserted in the vial at medium height to avoid excessive pressure increase during digestion. Another capillary was inserted through the septum to the base of the vial to incorporate reagents of MW-AD and HG. The digestion process was performed as follows: first, 2.5 mL of 2.4 M nitric acid (Pump P1; Valve V1, HNO₃) and then 2.5 mL of 30% (m v⁻¹) hydrogen peroxide (Pump P1; Valve V1, H₂O₂) for 2 minutes at a flow rate of 2.5 mL min⁻¹

were incorporated. After this valve V1 was closed, generating an airtight atmosphere. The samples were irradiated according to the following power program: 700 W, 13 seconds; 1000 W, 8 seconds; and 1500 W, 5 sec. After this, digests were allowed to cool for 5 minutes. Once digestion is completed, 1 mL of 6 M hydrochloric acid for 0.1 minutes at a flow rate of 5 mL min⁻¹ (Pump P1; Valve V1, HCl) was added for total selenium reduction.

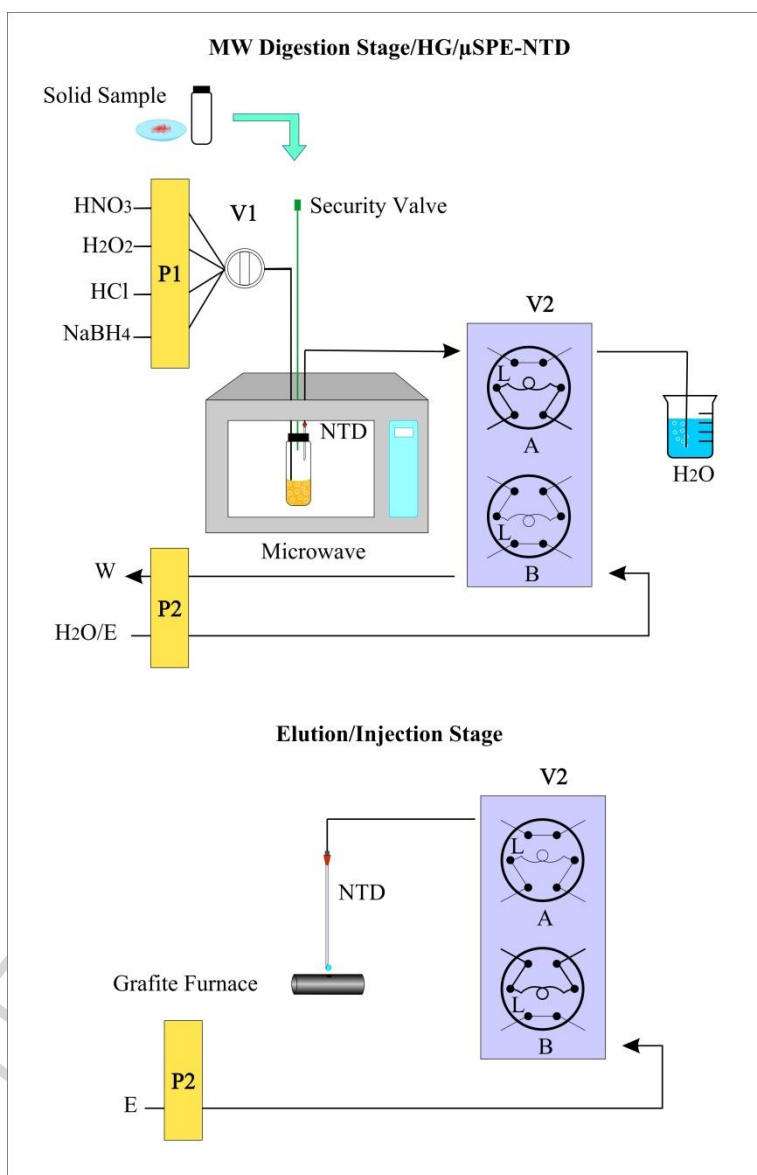


Figure 2: Schematic diagram instrumental setup. MW Digestion Stage/HG/NTD: E, eluent; V1 and V2, injection valves, valve positions: (A) hydride loading; (B) injection; L, loop. Elution/Injection stage: V2: injection valve; E: eluent.

In a second stage (HG and PRECONCENTRATION), the NTD was inserted through the septum and placed in the vial. The security valve was removed. Valve V2 was placed in the loading position (A) and 2.3 mL of 1% (m v⁻¹) NaBH₄ for 2 minutes at a flow rate of 2.5 mL min⁻¹ was aggregate (Pump P1; Valve V1, NaBH₄). During this step, the selenium hydride was formed and adsorbed in the NTD filled with oxMWCNTs. Loop L in Valve V2 was filled with the eluent (Pump P2).

Immediately, after completing HG, the NTD device, see the Figure 2 (Injection stage) with the retained Se, was mounted on the robotic arm of ETAAS autosampler in “stand by” position. Elution was achieved by setting the autosampler arm to “injection” position, and the NTD was inserted in the graphite tube with Iridium permanent modifier. The elution step was achieved with eluent E, 30 µL of 5% (m v⁻¹) HNO₃ (Pump P2 position B, valve V2 elution), for 0.1 minutes a flow rate of 0.5 mL min⁻¹.

3. Results and discussion.

3.1. Selenium atomization.

Atomization of selenium in graphite furnace shows many problems. Interferences occur on the surface, as well as in the gas phase, reducing drastically the analytical signal [25]. These selenium atomization problems increase when acidic solutions, as the one used for elution, are introduced into the graphite furnace. Under these conditions Ir was selected as a chemical modifier because it exhibited to be appropriate for Se determination in acidic solutions [26]. Ir permanent chemical modifiers have been shown to prolong graphite tube lifetime while reducing the furnace cycle time, thus improving cost-effectiveness [27].

Selenium can volatilize during pyrolysis stage. In the proposed methodology, the sample matrix does not reach the graphite furnace since only Se hydrides are adsorbed on oxMWCNTs. Accordingly, a graphite furnace program without pyrolysis was studied and no differences in Se signal were observed. In this way, the graphite furnace temperature program was shortened, becoming attractive in view of analytical frequency [28-30].

3.2. Multivariate optimization of NTD variables.

In order to select and optimize the variables of the proposed methodology, a multivariate design was implemented to achieve maximum sensitivity in the determination of selenium ultratraces by ETAAS. First, a screening step was carried out to select the significant variables in the sensitivity of the proposed methodology using an experimental Plackett Burman design [31]. Based on previous

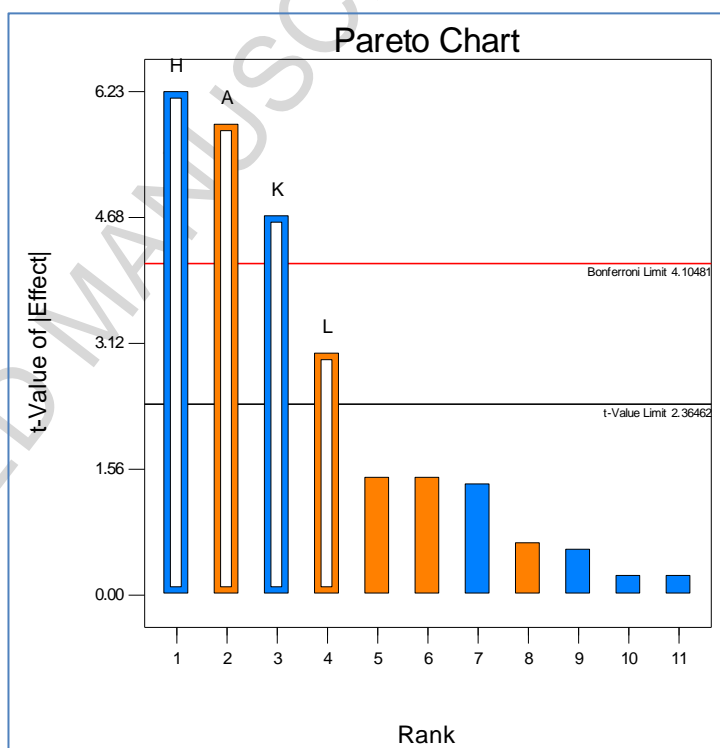


Figure 3: Pareto graph used to determine significant factors. A: Sample amount, H: elution volumen, K: NaBH₄ volume and L: HNO₃ concentration. The orange bars represent the positive effects and the blue bars the negative effects.

work [32, 33] and trials, eleven parameters were selected for evaluation within specific ranges that could significantly influence selenium micropreconcentration. These parameters were sample amount (100-300 mg), HNO₃ digestion solution concentration (2-3 M), HNO₃ digestion solution volume (2-3 mL), H₂O₂ concentration (30-70 %), H₂O₂ volume (2-3 mL),

HCl concentration (6-12 M), HCl volume (0.5-1.5 mL), NaBH₄ concentration (1-3 %), NaBH₄ volume (0.5-2.5 mL), NaBH₄ flow rate (0.25 - 2.5 mL min⁻¹) and elution volume (20-30 μL min⁻¹) while the measured response was the peak area absorbance. In this way, 13 experiments were performed with a central point. The Pareto graphic obtained (Figure 3) indicated that the following variables significantly affect the system: sample amount, HNO₃ digestion solution concentration, NaBH₄ volume and elution volume. The selected variables were evaluated by analysis of variance (ANOVA) obtaining a significant model for the response and a high R-Squared (0.9369) which shows a good relationship between the experimental data and the adjusted model.

The final optimization of the proposed methodology and the expected response according to the selected factors was carried out using the response surface method (RSM). A Box-Behnken design was implemented to determine the values at which the sensitivity measured from the absorbance is maximum [34]. A total of 29 experiments were performed with 5 central points in random order from this design. For this stage of experiments, the ranges of the variables to be studied were kept in the same ranges as in the screening step. On the other hand, the factors that remained fixed were maintained in the following values: 2.5 mL HNO₃ digestion solution volume, 30% H₂O₂ concentration, 2.5 mL H₂O₂ volume, 6M HCl concentration, 1 mL HCl volume, 1% NaBH₄ concentration and 2.5 mL min⁻¹ NaBH₄ flow rate. Experimental data showed good agreement as a second-order polynomial model. According to the ANOVA result, the model F-value of 64.05 implies that the model is significant. There is only 0.01% probability that the "Model F-Value" of this size can occur due to noise. R-square and adjusted R-squared (with values 0.9846% and 0.9693%, respectively) show a good correlation between experimental and theoretical results. It has been expressed that a model with a good fit should be at least 0.8. Depending on the results

obtained, where the coefficient of determination, R^2 , is > 0.8 , it can be stated that the equation obtained is adequate to correlate the experimental results [33].

The response surfaces (RSMs) shows the interaction between two selected factors determining the optimal conditions to obtain the maximum absorbance. Figure 4 and 1S (Supplementary Material) shows the 3D RSMs response obtained for some possible configurations fixing two factors and varying the other two factors. Figure 4A graphs the response surface calculated as a function of the interaction of the HNO_3 digestion solution concentration and the sample amount, leaving the other two variables in constant values. It can be observed that the optimum values are obtained by decreasing the sample amount and nitric acid concentration during digestion. This observation can be explained considering that an increase in sample quantity and acid concentration, generates a pressure increase inside the headspace vial, causing during the preconcentration stage a sudden gas release, decreasing the interaction of selenium hydrides with the NTCs in the needle. In addition, since HCl is used for prereduction of Se(VI) to Se(IV), strong oxidizing agents such as HNO_3 (or nitrogen oxides) and H_2O_2 , which are used during sample digestion, can oxidize HCl to Cl_2 and cause back-oxidation of Se(IV) during the prereduction step.

Figure 4B, shows how the NaBH_4 optimum volume is achieved by increasing this to near maximum values. This could be due to the fact that sodium tetrahydroborate not only provide the hydrogens to generate the selenium hydrides, but would be the responsible for generating the pressure increase in the vial headspace that allows the adequate flow of the gas to achieve the optimal interaction of the selenium hydrides with the NTCs.

Figure 4C shows the interaction between eluent volume and nitric acid volume. The elution efficiency increases with higher elution volumes, inferring that a minimum of 30 μL is needed to achieve a quantitative release of selenium hydrides from NTD. Selenium hydride can derive in its protonated species, in acidic conditions, called selenium (H_3Se^+) [35]. This univalent cation is electrostatically adsorbed by the carboxyl groups generated on the surface of the carbon nanotubes. Then, in the elution stage, an ion exchange takes place, between the species in question and the protons contributed by the acid eluent.

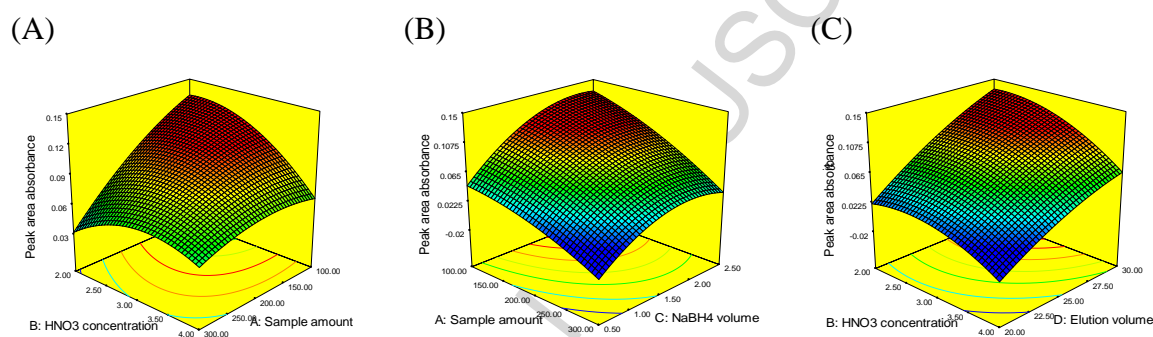


Figure 4: Response surfaces for: (A) Sample amount – HNO_3 digestion solution concentration, (B) Sample amount – NaBH_4 volume and (C) HNO_3 digestion solution concentration – Elution volume.

One of the outstanding advantages of the proposed methodology, is the combination of analytes preconcentration in gaseous phase with minimal reagents consumption compared to liquid SPE systems [36]. In addition, there are not backpressure problems, which are very common in liquid SPE systems employing CNTs as sorbent [37].

The optimal conditions shown by the model were: sample amount 100 mg, concentration of 2.4 M HNO_3 digestion solution, volume of NaBH_4 2.3 mL and volume of elution of 30 μL . Subsequently, an experimental confirmation of these optimal conditions was performed, where the response was compared with that predicted by the model and no significant difference was observed at a 95% confidence level.

3.3. Analytical Performance.

Under optimum conditions (Table 1), the proposed methodology showed a linear calibration curve with a correlation coefficient of 0.9975 from levels close to the quantification limit (LOQ) up to at least $10 \mu\text{g kg}^{-1}$. The NTD retention at these concentration levels was of 98.8%. The LOQ was calculated as 10 times the amount of Se required to produce a net peak that was equal to three times the standard deviation of the background signal (10σ , $n=3$) obtaining a value of $0.11 \mu\text{g kg}^{-1}$. Detection limit (DL), defined as the amount of analyte required to produce a net peak equal to three times the standard deviation of the background signal (3σ , $n = 3$) was $0.03 \mu\text{g kg}^{-1}$. Precision was assessed by relative standard deviation (RSD %) corresponding to 4% ($n = 3$) [38].

Table 1: Experimental conditions for Se determination in grape pomace.

Parameter	Value
Sample amount	100 mg
HNO ₃ digestion solution concentration	2.4 M
HNO ₃ digestion solution volume	2.5 mL
H ₂ O ₂ concentration	30 %
H ₂ O ₂ volume	2.5 mL
HCl concentration	6 M
HCl volume	1 mL
NaBH ₄ concentration	1 %
NaBH ₄ volume	2.3 mL
NaBH ₄ flow rate	2.5 mL min^{-1}
Elution volume	30 μL

The preconcentration factor (PF) was calculated as the ratio of the final concentration of analyte in the eluent phase and the concentration in the sample under the optimum conditions [39]. In order to calculate the PF of the analyte, preconcentration replicates were performed under optimum conditions from the sample containing $4 \mu\text{g kg}^{-1}$ of the analyte, therefore, the calculated PF corresponds to 100.

The time of analysis was calculated as follows: the time required for the MW-AD of 100 mg of sample (2 minutes, at a flow rate of 2.5 mL min⁻¹ for the addition of HNO₃ digestion solution and H₂O₂ and 6 minutes to implement the microwave power program), preconcentration (0.1 minute at a flow rate of 5 mL min⁻¹ for HCl addition and 2 minutes at a flow rate of 2.5 mL min⁻¹ for NaBH₄ addition), elution (0.1 min, at a flow rate of 0.5 mL min⁻¹), washing and conditioning (0.3 min), and atomization (0.9 min) was approximately 11 min, reaching a throughput sample of 5 samples per hour.

In Table 2, it is possible to observe a comparison between some characteristics of this technique and others that use treatment of an organic sample and determination of the analyte by atomic absorption spectrophotometry. The developed methodology presents lower LOQ and LD, with lower sample consumption and consequently a lower use of reagents and a significant reduced time of analysis.

Table 2: Comparison of methodologies AAS introducing Se determination in organic samples.

Sample	Sample amount	Sample treatment	Extraction technique	Analysis time	LD	LOQ	Reference
Food	250 mg	UV-photolysis	Evaporation	>120 minutes	1.2 µg L ⁻¹	4.0 µg L ⁻¹	[40]
Food	1000 mg	Microwave digestion	Vortex-assisted ionic liquid dispersive - microextraction	>120 minutes	0.098 µg L ⁻¹	-	[41]
Vegetables	500 mg	Microwave digestion	Activated carbon (AC) separation	>120 minutes	0.5-1.3 µg L ⁻¹	1.7-3.3 µg L ⁻¹	[42]
Food	1000 mg	Wet digestion	-	>120 minutes	2.0–7.0 µg kg ⁻¹	22 µg kg ⁻¹	[43]
Grape pomace	100 mg	Microwave digestion	NTD	11 minutes	0.03 µg kg ⁻¹	0.11 µg kg ⁻¹	This work

3.4. Validation and application.

Since there is no certified reference material (CRM) with similar characteristics or Se concentration range to the sample used in the present study, the standard addition method and comparison with other techniques were implemented to determine at least the precision of the proposed methodology. Thus, 3 aliquots of grape pomace sample were analyzed by the proposed method, setting the selenium concentration founded as base value. Increasing concentrations of Se standard solution were added to three other aliquots. The recovery was then calculated and results are shown in the Table 3. Recoveries were between 98.3 ± 9.5 and $102.9 \pm 9.2\%$.

Table 3: Recovery study and digestion validation.^c

Samples	Base value ($\mu\text{g kg}^{-1}$)	Added Se ($\mu\text{g kg}^{-1}$)	Found Se ($\mu\text{g kg}^{-1}$)	Recovery (%)
1	-	0	0.48 ± 0.01	-
2	0.48	1	1.51 ± 0.05	102.9^a
3	0.48	3,5	3.92 ± 0.12	98.4^a
4	0.48	5	5.45 ± 0.15	99.4^a
1 ^b	-	0	0.48 ± 0.01	-
2 ^b	0.48	1	1.49 ± 0.04	101.3^a
3 ^b	0.48	3,5	3.92 ± 0.1	98.4^a
4 ^b	0.48	5	5.53 ± 0.14	101.0^a

^a $\text{Se } 100 \times (\text{found} - \text{base}) / (\text{added})$.

^b Analysis performed with a laboratory microwave digester.

^c Confidence intervals: $t_{(0.05,df)} S / (n)^{0.5}$. Informed values as average amounts of three replicate determinations ($n = 3$).

To evaluate the method accuracy regard sample digestion results obtained with the proposed methodology were compared by MW-AD in closed vessels, with a laboratory microwave digester. This comparison results appropriate considering possible Se losses during the open vessel MW-AD in a domestic microwave digester developed in this

manuscript. Results can be observed in Table 3. The determined selenium concentrations show no significant difference at 95% confidence level. It has been stated that As and Bi cause Se HG interferences [44]. These elements, at the concentration range expected in the analyzed samples ($0.1 - 10 \mu\text{g kg}^{-1}$) showed no interferences.

The analysis of grape pomace used for the wine industry of the Tulum Valley, San Juan, Argentina, provided a range of concentrations between $0.34 \pm 0.03 \mu\text{g kg}^{-1}$ to $0.48 \pm 0.03 \mu\text{g kg}^{-1}$ for a total of 30 samples determinates. Although these concentrations do not represent a risk, considering that selenium content in most soils is very low, between $0.01-2 \text{ mg kg}^{-1}$ (the world average is 0.4 mg kg^{-1}) [45], it is important to emphasize the importance of controlling the use of marc pomace as fertilizer. Because on one hand, Se can be adsorbed on the organic matter generating deficiencies of the element. On the other hand, when it is used in large quantities in crop soil, selenium leaching may occur if the irrigation water presents an acidic pH with subsequent contamination of groundwater by accumulation of selenium over time [46]. This is most important when selenium tolerance limits in water reported by the US Environmental Protection Agency (EPA) correspond to only $50 \mu\text{g L}^{-1}$ [47].

4. Conclusion.

This research described a miniaturized methodology that integrates MW-AD, HG and NTD in FI system for determination of selenium in grape pomace samples. The proposed methodology allowed minimal reagents and sample consumption. Thus, the

preparation of the sample was much simpler and faster than conventional methods, reducing the probability of contamination by sample manipulation.

The NTD filled with oxidized MWCNTs was used for the first time in the preconcentration of selenium gaseous hydrides, presenting an alternative to the conventional hydride entrapment method. By means of a multivariate study it was possible to set the optimum conditions of several variables affecting the proposed methodology reaching a quantitative adsorption under the working concentrations range.

The system was successfully coupled to an FI system and ETAAS for selenium determination. The low eluent volume required for Se release from oxidized MWCNTs encompassed with the graphite furnace volume. Detection limits obtained by this configuration reach the nanogram level, a concentration range compatible with selenium concentrations in pomace samples.

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Appendix A. Supplementary data.

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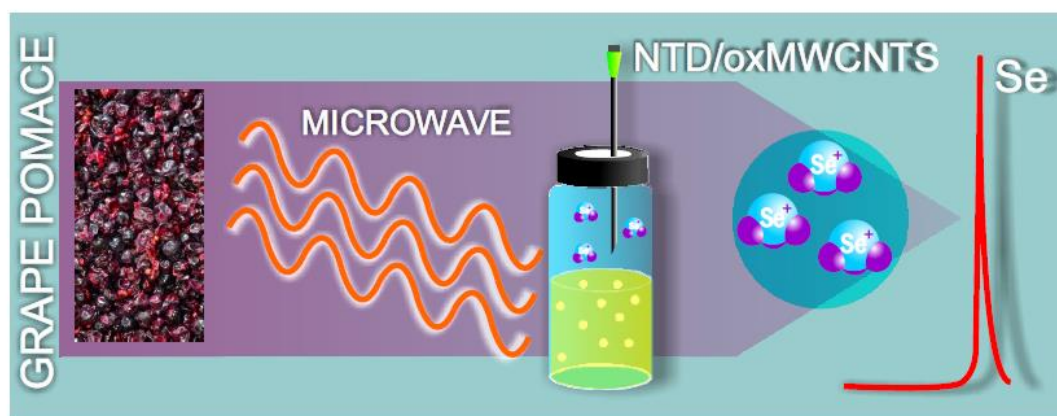
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Graphical abstract



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Highlights

- Pomace samples were digested by FI-MW-AD for Se analysis.
- A needle trap device filled with oxidized carbon nanotubes is presented.
- Se hydrides are successfully trapped on oxidized multiwall carbon nanotubes.
- The system is miniaturized with minimal samples and reagents consumption.
- Detection limits obtained by this configuration reach the nanogram level.

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